Insecticides and Their Combinations Evaluated as Regulatory Immersion Treatments for Third-Instar Japanese Beetle (Coleoptera: Scarabaeidae) in Field-Grown and Containerized Nursery Plants[1]

J.B. Oliver[2], C.M. Ranger[3], M.E. Reding[3], N.N. Youssef, and J.J. Moyseenko[3]

Tennessee State University, College of Agriculture, Human and Natural Sciences, Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, Tennessee 37110 USA

J. Entomol. Sci. 52(3): 274–287 (July 2017)

Abstract Japanese beetles, *Popillia japonica* Newman, are nursery regulatory pests. Currently, immersion of balled-and-burlapped (B&B) and containerized plants grown in pine bark substrates in a chlorpyrifos or bifenthrin solution satisfies the Domestic Japanese Beetle Harmonization Plan (DJHP) for shipping plants to noninfested states. Study objectives were to (a) evaluate individual and combination insecticide treatments for potential as regulatory dips against third-instar *P. japonica* in 30-cm B&B and no. 3 containers and (b) determine the lowest effective rates. Tests were performed fall and spring from 2007 to 2010. In all B&B tests and most container tests, insecticide treatments had significantly fewer larvae than the untreated check. Treatments also were more effective during spring tests than fall tests. The highest rate of a bifenthrin + imidacloprid combination was the only treatment that consistently met the DJHP regulatory standard of no larvae recovered across multiple tests. During spring tests with B&B and container plants, all rates tested of bifenthrin, bifenthrin + carbaryl, chlorantraniliprole, clothianidin, or dinotefuran met the no-larval-recovery DJHP standard. The lowest effective bifenthrin rate during spring tests was 9× lower than the current DJHP bifenthrin dip rate. Several treatments in this study met DJHP regulatory standards for dipping B&B and containerized plants and during spring timings bifenthrin alone or in combination with carbaryl or imidacloprid was effective at rates lower than currently allowed in the DJHP.

Key Words *Popillia japonica*, nursery, insecticide, scarab, plant dip

The Japanese beetle, *Popillia japonica* Newman, is a regulated pest in the United States affecting shipments of nursery plants to certain states. The adult stage is damaging to numerous crop and ornamental plants and larvae are important turf pests with an estimated $616 million/yr impact (Fleming 1972, USDA Animal and Plant Health Inspection Service 2007). The movement of the adult stage is regulated by a federal quarantine (Potter and Held 2002), but larval movement in nursery plants and grass sod is governed by the National Plant Board U.S. Domestic Japanese Beetle Harmonization Plan (DJHP) (National Plant Board 2017). The DJHP classifies states into four categories based on *P. japonica*

[1]Received 07 February 2017; accepted for publication 20 April 2017.
[2]Corresponding author (email: joliver@tnstate.edu).
[3]USDA–Agricultural Research Service Application Technology Research Unit, Horticultural Insects Research Laboratory, 1680 Madison Ave., Wooster, Ohio 44691 USA.

infestation status. Category 1 states (mostly west of the Rocky Mountains) are presently uninfested and considered high risk for agricultural impact if the beetle is introduced; thus, nursery plant shipping requirements to these states are the most stringent. Category 2 states are considered uninfested or partially infested and are primarily in the central United States. Category 3 states are primarily in the eastern United States and are considered infested with *P. japonica* and Category 4 states (currently Florida, Louisiana, and Wyoming) are historically not known to be infested and considered of no regulatory significance.

Nursery certification requirements for shipping plants from Category 3 to Category 2 states are less strict than requirements for shipping to Category 1 states. At the present time, nursery plants grown in field soil can be certified for Category 1 states only if shipped without soil (i.e., bare root), but two treatments are approved for treating the roots and soil of field-grown plants shipped to Category 2 states. The two approved treatments for field-grown nursery stock include a preharvest soil surface spray of various imidacloprid formulations including imidacloprid + cyfluthrin (Discus® N/G; OHP, Inc., Mainland, PA) or thiamethoxam (Flagship® 25WG or 0.22G; Syngenta Crop Protection, Inc., Greensboro, NC) during May through July, or a postharvest immersion (dip) of balled-and-burlapped root balls (B&B) in bifenthrin or chlorpyrifos (Oliver et al. 2009, 2013, 2016; National Plant Board 2017). Containerized nursery plants grown in soilless substrates like pine bark also can be dipped in bifenthrin or chlorpyrifos, but unlike field-grown plants, containers can be certified for either Category 1 or 2 states (if the container diameter is <30 cm for Category 1 states). In addition to the dip option, container-grown plants can be drenched in bifenthrin, imidacloprid, imidacloprid + cyfluthrin, or thiamethoxam, or granular bifenthrin or imidacloprid can be incorporated into the container substrate (National Plant Board 2017). The rationale for allowing container shipments to Category 1 states using a larger range of active ingredients and treatment methods include the low propensity for *P. japonica* to infest and survive in container substrates (Smitley 1994), and the greater insecticide movement and penetration in porous container substrates (Simmons and Derr 2007).

Dip treatments are the least preferred technique by growers for treating B&B or container-grown nursery plants. However, in the case of field-grown plants, producers that fail to apply the preharvest soil treatment only have the dip option. Likewise, container producers that have not incorporated insecticide into the planting substrate are restricted to either the drench or dip option. The dip option is presently limited to either chlorpyrifos (30.0 g active ingredient [AI]/100 L) or bifenthrin (27.0 g AI/100 L) (hereafter, amounts given in grams of active ingredient will refer to AI/100 L). Dip treatments are more effective at controlling third-instar *P. japonica* than methods such as drenching, soil injection, or surface sprays (Mannion et al. 2000, 2001; Oliver et al. 2007, 2008). Because dip treatments are more efficacious than other application methods, dip testing has some value for determining insecticides not likely to work by other methods and for determining potential effective rates. For growers, the primary limitations of the dip method are the higher labor costs, hazards to labor, dip solution disposal, disruption of nursery plant soil and roots, and phytotoxicity.

To reduce some of the limitations associated with the dip method, one solution would be to provide lower-cost or reduced-risk insecticide options. Active

ingredients not currently labeled for dipping or approved for use as DJHP dips, but which have shown experimental efficacy as dips against *P. japonica* (as well as imported fire ants [*Solenopsis* spp.]), include acephate, carbaryl, deltamethrin, halofenozide, imidacloprid, thiamethoxam, and trichlorfon (Callcott et al. 2012, James et al. 2005, Klein et al. 2002, Oliver et al. 2016). Some of these potential alternatives have less acute human toxicity, have lower environmental hazard, are not restricted-use products, and have signal words of caution rather than warning (Calvert et al. 2004, Crop Data Management System 2017, Environmental Protection Agency 2002). Reducing the active ingredient rate also may mitigate some of the chemical hazards of dip treatments. Combinations of two insecticides also can result in greater insecticidal activity than when the products are used alone (synergistic or additive effects), potentially allowing the use of lower rates for both products (Bynum et al. 1997, Wilkinson 1976). The objectives of this study were to (a) evaluate individual and combination insecticides as regulatory dip treatments to eliminate third-instar *P. japonica* in small B&B nursery plants (~30 cm diameter) and container-substrates with grass (Poaceae) plants and (b) determine the lowest effective rates for some of the insecticides evaluated.

Materials and Methods

General test procedures. Multiple insecticide active ingredients were evaluated as regulatory dip treatments to control third-instar *P. japonica* infested into B&B or container substrates (Table 1). Insecticides and rates were selected for evaluation based on efficacy potential from previous work (Oliver et al. 2016). Tests were performed fall and spring from 2007 to 2010 (Table 2). Depending on efficacy, rates of some insecticides were lowered in subsequent tests. All B&B plants were obtained from nurseries in Warren County, TN where silt loam to cherty silt loam soils with bulk densities ranging from 1,537.6 to 1,761.9 kg/m^3 are the most common field soils (Jackson et al. 1967). For B&B testing, some tests had different nursery plants depending on availability, including burning bush (*Euonymus alatus* [Thunberg] Siebold variety 'Compactus') (fall 2007, fall 2008, spring 2009 tests), red maple (*Acer rubrum* L.) (spring 2008 test), sweet mock orange (*Philadelphus coronarius* L.) (fall 2009 test), and rose-of-Sharon (*Hibiscus syriacus* L.) (spring 2010 test). The B&B plants were harvested as approximately 30-cm-diameter root balls and wrapped in burlap using standard nursery methods. Although the complete insecticide application history for foliar pests at the B&B harvest sites was unknown, no recent regulatory soil treatments had been applied for soil-borne pests. Fertilization history also was unknown for B&B sites. For container tests, 11.7-L black-colored plastic containers (27-cm-diameter top and 25-cm height) (no. 3 HPP F300 Series Haviland Plastic Products, Haviland, OH) were filled to just below the top edge with Morton's Nursery Mix (Morton's Horticultural Suppliers, McMinnville, TN; 55–65% processed pine bark, 20% Canadian sphagnum peat, and 20% sand with a manufacturer reported bulk density of 191.0 kg/m^3) (2007 and 2008 tests) or Pro-Gro Mix (Barky Beaver, Moss, TN; 78% pine bark, 12% peat moss, 10% sand, and 4.8 kg lime/m^3 with a manufacturer reported bulk density range of 240.3 to 256.3 kg/m^3) (2009 and 2010 tests). Container substrates had no insecticide treatments other than those applied in experiments. Because containers

Table 1. Trade and common insecticide names, active ingredients, labeled rates, and manufacturers for insecticides used in balled and burlapped root ball and container-grown dip studies.

AI (% in product)*	Insecticide Trade Name**	Maximum Use Amount, g AI/ha per year†	Company‡
Bifenthrin EC (23.4)	OnyxPro® Insecticide	224.2	FMC
Bifenthrin F (7.9)	Talstar® Nursery F	224.2	FMC
Bifenthrin (4) + Imidacloprid (5)	Allectus® SC	1020.0	Bayer
Carbaryl (43)	Sevin® SL	22,416.6	Bayer
Chlorantraniliprole (18.4)	Acelepryn™ Insecticide	560.4	Dupont
Clothianidin (50)	Arena® 50WDG	448.3	Arysta
Cyfluthrin (0.7) + Imidacloprid (2.9)	Discus® N/G	694.9	OHP
Dinotefuran (20)	Safari® 20SG Insecticide	605.3	Valent
Imidacloprid (21.4)	Marathon® II	448.3	OHP
Trichlorfon (80)	Dylox 80 T&O Insecticide	27,460.3	Bayer

* AI = active ingredient. Chemical class of insecticides includes anthranilic diamide (chlorantraniliprole), neonicotinoid (clothianidin, dinotefuran, imidacloprid), pyrethroid (bifenthrin, cyfulthrin), pyrethroid + neonicotinoid (Allectus SC, Discus N/G), carbamate (carbaryl), and organophosphate (trichlorfon).
** Acelepryn Insecticide was an experimental (DPX-E2Y45) when tested and is now sold by Syngenta Crop Protection, Inc., Greensboro, NC. Arena is now sold by Valent U.S.A. Corporation, Walnut Creek, CA. For Dylox, T&O refers to Turf and Ornamental.
† Talstar Nursery Flowable is currently unavailable, but the nursery-labeled alternative (Talstar® S Select Insecticide) has the same formulation and rate requirements.
‡ Arysta, Arysta LifeScience North America, Cary, NC; Bayer, Bayer Environmental Science, Research Triangle Park, NC; Dupont, Dupont Professional Products, Wilmington, DE; FMC, FMC Corporation, Philadelphia, PA; OHP, OHP, Inc., Mainland, PA; and Valent, Valent U.S.A. Corporation, Walnut Creek, CA.

with grasses (Poaceae) or sedges (Cyperaceae) are more likely to have *P. japonica* larvae (Smitley 1994), each container received an approximately 30-ml volume of grass seed mixed into the top 5 cm of substrate to provide roots for larvae. Grass was growing in container plants at the time of larval introductions. During fall 2007 to spring 2009 tests, grass seed was a 1:1 mixture of tall fescue (*Festuca arundinacea* Schreber) and perennial ryegrass (*Lolium perenne* L.). During fall 2009 and spring 2010 tests, grass seed was a 2:1:1 mixture of tall fescue, perennial ryegrass, and Kentucky bluegrass (*Poa pratensis* L.). To prevent larval escape from containers or excess washing of substrate from drain holes during dipping, a piece of weed fabric barrier was placed in the bottom of each container to cover drain holes before substrates were added. All B&B treatments had 10 single-plant replications, while container plants had five replications. Container and B&B plants were arbitrarily

Table 2. Mean (± SE) third-instar Japanese beetle in 30-cm-diameter balled-and-burlapped (B&B) root balls with field soil and 27-cm-diameter containers (no. 3 size) with pine bark substrate and grass seed dipped in various insecticides and rates in the fall and spring of multiple years.

		Percentage Control of Third Instar Japanese Beetle (Total No. Live Larvae)†					
		B&B Plants ($n = 10$)					
Active Ingredient	Rate*	Fall 2007	Fall 2008	Fall 2009	Spring 2008	Spring 2009	Spring 2010
Bifenthrin F	27.6	100a (0)	100a (0)	97a (1)	100a (0)	100a (0)	100a (0)
	13.8	100a (0)	100a (0)	94a (2)	100a (0)	100a (0)	100a (0)
	6.9						
Bifenthrin EC	6.0	100a (0)	100a (0)	97a (1)	100a (0)	100a (0)	100a (0)
	4.5			97a (1)			100a (0)
	3.0						
Bifenthrin F + Carbaryl	1.5 + 30.0	100a (0)		97a (1)	100a (0)		100a (0)
	0.7 + 15.0		97a (1)	97a (1)		100a (0)	100a (0)
Bifenthrin F + Imidacloprid	24.0 + 30.0	100a (0)			100a (0)		
	24.0 + 30.3**	100a (0)			100a (0)		
	12.0 + 15.0		100a (0)			100a (0)	
	12.0 + 15.2**		100a (0)	100a (0)		95a (1)	100a (0)
	6.0 + 7.5		100a (0)			100a (0)	
	6.0 + 7.4**			100a (0)			100a (0)
	3.0 + 3.7			94a (2)			100a (0)
	1.5 + 1.9			94a (2)			100a (0)
Bifenthrin F + Trichlorfon	1.5 + 30.0	100a (0)			100a (0)		
	0.7 + 15.0		100a (0)	97a (1)		91a (2)	100a (0)
Chlorantraniliprole	50.0	88a (2)			100a (0)		
Clothianidin	48.0	100a (0)	90a (4)		100a (0)	100a (0)	
	24.0	94a (1)			100a (0)		
Cyfluthrin + Imidacloprid	5.3 + 22.6	100a (0)	92a (3)	88a (4)	100a (0)	95a (1)	
Dinotefuran	64.7	88a (2)			100a (0)		
	32.4						

Table 2. Extended.

Percentage Control of Third Instar Japanese Beetle (Total No. Live Larvae)†					
Container Plants ($n = 5$)					
Fall 2007	Fall 2008	Fall 2009	Spring 2008	Spring 2009	Spring 2010
100a (0)	100a (0)	100a (0)	100a (0)	100a (0)	100a (0)
100a (0)	100a (0)	100a (0)	100a (0)	100a (0)	100a (0)
	100a (0)	100a (0)		100a (0)	100a (0)
100a (0)	100a (0)	100a (0)	100a (0)	100a (0)	100a (0)
		100a (0)			100a (0)
	100a (0)	100a (0)		100a (0)	100a (0)
100a (0)	100a (0)	100a (0)	100a (0)	100a (0)	100a (0)
	100a (0)	100a (0)		100a (0)	100a (0)
100a (0)	100a (0)	100a (0)	100a (0)	100a (0)	100a (0)
	100a (0)	100a (0)		100a (0)	100a (0)
		100a (0)			100a (0)
100a (0)	100a (0)	100a (0)	86a (1)	100a (0)	100a (0)
	100a (0)	67a (1)		100a (0)	100a (0)
		100a (0)			100a (0)
100a (0)	88a (1)	67a (1)	100a (0)	100a (0)	100a (0)
	88a (1)	100a (0)		100a (0)	100a (0)

Table 2. Continued.

Active Ingredient	Rate*	Percentage Control of Third Instar Japanese Beetle (Total No. Live Larvae)† B&B Plants ($n = 10$) Fall 2007	Fall 2008	Fall 2009	Spring 2008	Spring 2009	Spring 2010
Nontreated check	0.0	—b (16)	—b (38)	—b (34)	—b (15)	—b (21)	—b (31)
Nontreated check mean ± SE		1.6 ± 0.4	3.8 ± 0.4	3.4 ± 0.5	1.5 ± 0.5	2.1 ± 0.4	3.1 ± 0.4
c^2		53.7	140.4	119.9	52.8	71.5	127.2
df		12	10	12	12	10	12
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* Rates are grams of active ingredient (AI) per 100 L.

** Treatment was prepared by combining Talstar Nursery Flowable with Marathon II. Other bifenthrin F + imidacloprid treatments were Allectus SC.

† Percentage of control calculated as ((nontreated check mean − insecticide treatment mean)/nontreated check mean) × 100. Percentages within a column followed by different letters had numbers of third-instar Japanese beetle that were significantly different ($P < 0.05$) using a Generalized Linear Interactive Model (GLIM; Proc GENMOD) with a log link assuming a negative binomial distribution with means separated by least squares means. Values in parenthesis are the total number of third-instar Japanese beetle recovered from all nursery plant replicates. All plants were infested with five larvae before treatment (except the spring 2008 test, which received six larvae). Mean ± standard error (SE) values are provided for the nontreated check treatment. For insecticide treatments, B&B plants had means ranging from 0 to 0.4 (0–4 larvae) and SE from 0.0 to 0.2, and container plants had means ranging from 0.0 to 0.2 (0–1 larva) and SE from 0.0 to 0.2, respectively.

placed into treatment groups because random mixing had occurred during preparation or harvest, loading for transport, and unloading at the treatment site.

All third-instar *P. japonica* were collected from turf sites and local turf farms and held in the laboratory until use as previously described by Oliver et al. (2016). All larvae were held for at least 24 h before use to ensure no mechanical injuries had occurred during collection; sick or discolored larvae were discarded (Oliver et al. 2016). All single-plant replicates received five larvae per plant ($n = 25$ or 50 larvae total for each treatment performed with container or B&B plants, respectively) with the exception of the spring 2008 B&B test, which had five larvae added in November and one additional larva in March ($n = 60$ larvae total). For B&B tests, nursery plants were infested by stabbing holes through the burlap and adding larvae as previously described by Oliver et al. (2016). For container tests, five small depressions were made in the substrate of each plant and one larva was dropped into each hole and allowed to enter the substrate without further assistance. Containers were infested on 5 October, 13 October, or 23

Table 2. Extended Continued.

Percentage Control of Third Instar Japanese Beetle (Total No. Live Larvae)†					
Container Plants ($n = 5$)					
Fall 2007	Fall 2008	Fall 2009	Spring 2008	Spring 2009	Spring 2010
—b (9)	—b (8)	—a (3)	—a (7)	—b (10)	—a (4)
1.8 ± 0.7	1.6 ± 0.6	0.6 ± 0.4	1.4 ± 0.7	2.0 ± 0.7	0.8 ± 0.8
23.5	22.5	5.3	16.1	31.6	8.4
7	13	16	7	13	16
0.0014	0.0482	0.9944	0.0244	0.0027	0.9374

September (fall 2007, 2008, or 2009 tests, respectively) or 11, 9–11, or 3 March (spring 2008, 2009, or 2010 tests, respectively). The B&B plants also were infested on the same dates with the exceptions of the spring 2008 test that received five larvae on 19–20 November 2007 and one additional larva on 11 March 2008, fall 2009 test infested on 28 September 2009, and spring 2010 test infested 11 March 2010. All test plants were infested approximately 1–2 wk before treatment, except for the spring 2008 test. Insecticide dip solutions were prepared and the B&B soil and burlap or container substrate immersed for 1 min in 114-L trash cans as previously described in Oliver et al. (2016). Although the DJHP requires a 2-min dip time (National Plant Board 2017), a shorter 1-min dip was used in tests since the smaller plants ceased from bubbling during that time, and it was felt that thorough saturation had occurred. If efficacious, a shorter dip time also could provide a labor cost benefit for nursery operations. During the container dip process, a small quantity of bark and peat substrate did float from the upper substrate surface, but the grass growing in the substrate minimized loss, and the weed fabric barrier lining the inside of the container prevented any substrate loss through the container drain holes. No larvae were observed floating in the dip solution, and it was unlikely any larvae were lost from the small quantity of surface substrate that floated during the dip process. All container and B&B dip treatments were completed in a 1–2-d period on the 15, 21, or 19 October (fall 2007, 2008, or 2009 tests, respectively) or 15–16, 25, or 22–23 March (spring 2008, 2009, or 2010 tests, respectively). Due to the number of treatments and to avoid cross-contamination, dips were applied to only one or two treatments at a time. To avoid cross-contamination after treatment, plants were placed back into treatment groups until larval assessments were performed 147–149 d posttreatment on 11, 19, or 15–16 March (fall 2007, 2008, or 2009 tests,

respectively) or 52–62 d posttreatment on 16, 16, or 21–23 May (spring 2008, 2009, or 2010 tests, respectively). Fall test plants were covered with overwintering blankets in November or December when temperatures fell below freezing and were uncovered in late February or March when temperatures were above freezing. Spring test plants were watered every 2–3 d when transpiring plants increased watering needs, and fall tests plants were watered as needed. Larval assessments were performed by breaking plants apart and examining soil and bark substrates for surviving larvae. Natural infestations were possible in B&B plants, but plants were not preassessed for existing infestations, and it was assumed all plants had similar levels of natural infestation in addition to the *P. japonica* larvae artificially added. Natural infestations in the container plants were unlikely because plants were potted in the fall after the adult *P. japonica* flight season using container substrate from prepackaged bags. All scarab larvae were identified to at least genus using the raster pattern (Shetlar and Andon 2012). Phytotoxicity assessments were not performed in this study. A generalized interactive model (GLIM) (Proc GENMOD) using a log link, and assuming a negative binomial distribution, was used to compare larval numbers among treatments and means were separated using least squares means ($P < 0.05$) (Agresti 2002) (SAS 9.1.3 Service Pack 3. SAS Institute, Cary, NC). The GLIM procedure does not perform well for treatments that have all zero values, so a value of 0.5 was added arbitrarily to one replicate in this situation.

Results and Discussion

Insecticide dip treatments significantly reduced numbers of *P. japonica* larvae compared to water check treatments in all B&B tests (Table 2). For container tests, insecticide dips also significantly reduced *P. japonica* numbers compared to water check treatments in the fall 2007 and 2008 tests and the spring 2009 test (Table 2). No other significant differences were detected in other container tests, which likely was due to low larval recoveries in the check treatments of fall 2009 ($n = 3$), spring 2008 ($n = 7$), and spring 2010 ($n = 4$) tests (Table 2). In general, container tests had low larval recoveries (range of 3 to 10) relative to B&B tests (range of 15 to 38). The spring 2008 container test had a significant model effect, but mean separations did not identify differences between the check and insecticide treatment means.

Although most insecticide treatments had statistically fewer larvae than the untreated check, from a regulatory perspective, quarantine treatment efficacy is based on presence or absence of larvae and not an acceptable larval threshold number. The DJHP does not have an acceptable threshold for presence of *P. japonica* larvae in nursery plants or grass sod (National Plant Board 2017). The DJHP does define a larval threshold of ≤ 1 larva in the Nursery Accreditation Program (NAP), which is a field-sampling protocol to determine if larval populations at a site are low enough to permit certification of plants harvested from that localized area (National Plant Board 2017). The NAP criteria of ≤ 1 larva could potentially be used as a threshold to define the success of a research treatment, but a concern with an artificial research threshold would be how many plant replicates had to be free of larvae to be representative of a typical nursery shipment and to qualify as a successful treatment. In the present study, we used 10 B&B and 5 container

replicates, but it is doubtful these replicate numbers are even close to the typical number of plants shipped by commercial nurseries in a single load. Consequently, the best success measure for a regulatory treatment likely would be consistency of larval control across multiple tests over time. Likewise, if larvae were present at any insecticide treatment rate, then it also would be logical to assume inconsistency of control at that rate or any rates below that rate even if other tests sometimes had no larvae recovered at lower rates.

Using an acceptable regulatory criterion of no larvae found in any test at a given rate, only a few B&B dip treatments were satisfactory in this study (Table 2). The B&B treatments that would meet this level of efficacy across study tests were bifenthrin F + imidacloprid at 24 + 30 g AI or bifenthrin F + imidacloprid at 1.5 + 30 g AI, respectively (Table 2). If we broaden our acceptable criterion to $\leq$1 larvae, then other B&B treatments did meet this level of efficacy across tests, including bifenthrin F (27.6 g AI), bifenthrin EC ($\geq$4.5 g AI), bifenthrin F + imidacloprid at $\geq$6 + 7.4 g AI, or bifenthrin F + trichlorfon at 1.5 + 30 g AI, respectively. There were more treatments providing complete larval control across container tests, including all rates of bifenthrin F and EC, bifenthrin + carbaryl, bifenthrin + imidacloprid, and cyfluthrin + imidacloprid (Table 2). All remaining container treatments and rates met a $\leq$1 larva level of efficacy across tests (Table 2). With respect to test timing in B&B plants, all rates of bifenthrin F and EC, bifenthrin + carbaryl, chlorantraniliprole, clothianidin, and dinotefuran were 100% effective in the spring, but not the fall (Table 2). Likewise, almost all rates of bifenthrin F + imidacloprid were more effective in the spring than the fall in B&B plants (Table 2). For containers, most treatments had complete larval control whether fall or spring, but dinotefuran was one insecticide that also provided complete control in the spring at all rates tested, but not in the fall (Table 2).

The DJHP currently requires a bifenthrin rate of 26.9 g AI/100 L (National Plant Board 2017). The presence of larvae in some fall dip tests at the highest 27.6-g AI bifenthrin rate would support the need for the current DJHP bifenthrin rate. However, spring test results suggest bifenthrin alone may be efficacious in B&B and containers at rates as low as 3 g AI and down to 1.5 and 0.7 g AI when combined with imidacloprid or carbaryl, respectively (Table 2). The 0.7 and 3 g AI bifenthrin rates were 38$\times$ and 9$\times$ lower than the current DJHP rate, respectively. The flowable formulation of bifenthrin is not presently approved for DJHP use, and current labels do not support a dip use pattern (Crop Data Management System 2017, National Plant Board 2017). However, the flowable formulation of bifenthrin would likely be effective at the current DJHP rate based on consistent efficacy across tests in this study at the 27.6-g AI rate.

Container and B&B tests with trichlorfon and bifenthrin combinations had more variable *P. japonica* control across rates and seasons (Table 2), and these inconsistencies likely indicate combined rates of these insecticides were too low to meet DJHP standards. In a previous study, carbaryl or trichlorfon treatments were both 100% effective against *P. japonica* at spring B&B dip rates $\geq$7.5 g AI, but during the fall carbaryl only was effective at rates $\geq$30 g AI and trichlorfon at rates $\geq$479.4 g AI, respectively (Oliver et al. 2016). Consequently, in this study fall B&B dip combinations of carbaryl or trichlorfon with bifenthrin met DJHP standards at rates lower than when the products were tested individually. Imidacloprid applied individually as a B&B dip met DJHP level control at rates of $\geq$47.9 g AI across

spring and ≥24 g AI across fall tests (Oliver et al. 2016). Apparently the combination of imidacloprid and bifenthrin provided acceptable control of *P. japonica* at rates lower than when either insecticide was used alone.

With the exception of trichlorfon and bifenthrin combinations, spring B&B and container insecticide treatments were more effective than fall treatments. Higher spring *P. japonica* mortality also has been observed in other dip and drench studies (Oliver et al. 2008, 2016). The explanation for the apparent enhanced mortality during spring exposure periods is unknown, but differences in larval activity and physiological state are likely explanatory factors. At the mid- to late-October treatment timings of this study, *P. japonica* larvae are beginning to reduce their feeding and to move deeper into the soil profile to overwinter. In September, newly molted third instars are actively feeding and their mixed-function oxidase (MFO) system has very high gut tissue protein titers, but later in the year nonfeeding third instars, prepupae, and pupae have significantly lower MFO enzyme titers (Ahmad 1983). The MFO system is a general enzyme detoxification system present in the gut tissues, Malpighian tubules, and fat bodies of insects, which facilitates degradation of plant toxins and pesticides (Ahmad 1983). The low MFO activity in the spring could be a factor in the enhanced susceptibility of *P. japonica* larvae to insecticides. In addition to potential MFO effects, we also observed that fall-collected larvae had heavy yellow fat deposits visible below the integument, but these fat bodies were less apparent in spring larvae and probably were depleted during overwintering. The March period of our spring dip tests was not studied for MFO activity levels by Ahmad (1983), but third-instar *P. japonica* are actively feeding again at this time of the year. If larval insecticide toxicity occurs primarily via an oral route, then increased spring feeding relative to cessation in the fall would expose larvae to more insecticide in the spring at a time when MFO protective detoxification activity potentially is lowest. Likewise, if plants with fall-treated larvae had been evaluated in May rather than March, it is possible larval survival also might have been lower, especially for insecticides with long residual activity in the soil like bifenthrin (Nielsen and Cowles 1998). More research will be needed to determine if fall-treated larvae eventually would succumb to insecticide effects in late spring when MFO activity lowers, and therefore, would still meet regulatory efficacy requirements.

The dip application method was effective at meeting regulatory efficacy with several active ingredients, combinations, and rates, especially during spring tests. Likewise, regulatory-level efficacy across tests tended to be more consistent with container treatments than with B&B plants. The dip method also was effective in other studies (Mannion et al. 2000; Oliver et al. 2007, 2016). The dip technique resulted in fewer *P. japonica* larvae in field-grown nursery plants than other application methods, such as drenching, injection, and preharvest surface sprays (Mannion et al. 2000, 2001; Oliver et al. 2008, 2009). The effectiveness of the dip procedure relates to the thorough saturation of the root ball soil and increased exposure of larvae to insecticide treatments (Oliver et al. 2016). Likewise, greater consistency of the dip technique in container than B&B plants was likely a factor of the large void space and low bulk density characteristic of pine bark substrates, which increases hydraulic conductivity of solutions (Simmons and Derr 2007). It also is likely the container substrate environment is less suitable for larval establishment than field soil because *P. japonica* larvae are rarely found in

container substrates unless grass (Poaceae) or sedge (Cyperaceae) plants are present (Smitley 1994). The 1-min dip time in this study, which was shorter than the 2-min dip time required by the DJHP (National Plant Board 2017), was still sufficient to control *P. japonica* larvae with most B&B and container treatments. The 1-min dip also was effective for treating 30-cm root balls in another study (Oliver et al. 2016), but larger root balls (e.g., 60–80 cm diameter) with more soil volume required 2- and 5-min dips to be adequately treated (Mannion et al. 2000).

In summary, many B&B and container insecticide treatments had significantly fewer larvae than the check treatment, but many of these same treatments were still not satisfactory DJHP treatments based on the presence of larvae. Spring treatment timings clearly had greater efficacy in both B&B and containers, but using a regulatory standard of no larvae present, many fall treatments would not have qualified as DJHP treatments. Most bifenthrin rates, bifenthrin + carbaryl, and bifenthrin + imidacloprid met a no-larva level of control during the spring tests. More research will be needed to determine if fall treatment efficacy can be improved by evaluating test plants later in the spring. An added regulatory benefit of treatments with bifenthrin is the additional control provided on imported fire ants (Callcott et al. 2012). Overall, our current study found several insecticides with potential for *P. japonica* regulatory dip treatments for B&B or container nursery plants and that bifenthrin with carbaryl or imidacloprid combinations were effective against *P. japonica* at rates lower than when these products were used alone.

Acknowledgments

We thank Joshua Basham, Lorinda Long, Josh Medley, Samuel Ochieng, and Samuel Patton (Tennessee State University [TSU]) for providing assistance and several agrochemical manufacturers listed in Table 1 for providing product for testing. Partial research funding was provided by USDA-NIFA Evans-Allen, USDA-ARS Thad Cochran Southern Horticultural Research Laboratory, USDA-ARS National Arboretum, USDA-ARS Application Technology Research Unit–Horticultural Insects Research Laboratory, USDA-APHIS Biloxi Station, and the TSU College of Agriculture, Human, and Natural Sciences. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Tennessee State University or the U.S. Department of Agriculture.

References Cited

Agresti, A. 2002. Categorical Data Analysis. 2nd ed. John Wiley & Sons, Inc., New York.

Ahmad, S. 1983. Mixed-function oxidase activity in a generalist herbivore in relation to its biology, food plants, and feeding history. Ecology 64: 235–243.

Bynum, E.D., Jr., T.L. Archer and F.W. Plapp, Jr. 1997. Comparison of banks grass mite and twospotted spider mite (Acari: Tetranychidae): Responses to insecticides alone and in synergistic combinations. J. Econ. Entomol. 90: 1125–1130.

Callcott, A., X. Wei, S. James, L. McAnally, C. Hinton, J. Oliver, N. Youssef, C. Ranger, M. Reding and J. Moyseenko. 2012. Summary of balled-and-burlapped (B&B) immersion/dip treatments for potential use in the Federal IFA Quarantine Program: 2002–2010, Pp. 15–23. *In* USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine 2012 CPHST Laboratory Report, Gulfport / Biloxi Station. Biloxi, MS. 7 January 2017. (https://www.aphis.usda.gov/plant_health/plant_pest_info/fireants/downloads/Gulfport/2012_Annual_Report.pdf).

Calvert, G.M., D.K. Plate, D.R. Rosales, O. Shafey, C. Thomsen, D. Male, J. Beckman, E. Arvizu and M. Lackovic. 2004. Acute occupational pesticide-related illness in the US, 1998–1999; surveillance findings from the SENSOR-Pesticides Program. Am. J. Indust. Med. 45: 14–23.

Crop Data Management Systems. 2017. Crop Data Management Systems, Inc., Marysville, CA. 7 January 2017. http://www.cdms.net/Label-Database.

Environmental Protection Agency. 2002. Interim reregistration eligibility decision for chlorpyrifos. EPA-738-R-01-007 7508C.

Fleming, W.E. 1972. Biology of the Japanese beetle. USDA-ARS Technical Bull. No. 1449.

Jackson, G.T., S.R. Bacon, J.F. Brasfield, D.B. Freeman and I.D. Persinger. 1967. Soil survey of Warren County, TN. USDA Soil Conservation Service in Cooperation with Tennessee Agricultural Experiment Station.

James, S., J. Oliver, A. Callcott, M. Klein, J. Moyseenko, N. Youssef and L. McAnally. 2005. Immersion treatments for imported fire ant quarantine with consideration for Japanese beetle grubs, Pp. 49–52. *In* Weeks, R., A. Callcott, S. James, D.J. Meloche and M. Collins (eds.), Annual Red Imported Fire Ant Conference, 22–24 March 2005. Gulfport, MS.

Klein, M.G., J.B. Oliver, J.J. Moyseenko and M.E. Reding. 2002. Insecticidal dips and other strategies for elimination of Japanese beetle larvae from balled and burlapped nursery stock, Pp. 176–182. *In* Oliver, J.B. (ed.), Proceedings 47th Annual Southern Nursery Association Research Conference, 1–2 August 2002, Southern Nursery Association, Atlanta, GA.

Mannion, C.M., W. McLane, M.G. Klein, J. Moyseenko, J.B. Oliver and D. Cowan. 2001. Management of early-instar Japanese beetle (Coleoptera: Scarabaeidae) in field-grown nursery crops. J. Econ. Entomol. 94: 1151–1161.

Mannion, C.M., W. McLane, M.G. Klein, D.G. Nielsen and D.A. Herms. 2000. Insecticide dips for control of Japanese beetle and other soil-infesting white grubs in B&B nursery stock. J. Environ. Hort. 18: 89–93.

National Plant Board. 2017. US Domestic Japanese Beetle Harmonization Plan. 6 Jan. 2017. (http://www.nationalplantboard.org/wp-content/uploads/docs/jbhp_2017_final.pdf).

Nielsen, D.G. and R.S. Cowles. 1998. Preventing white grub infestation in container-grown nursery stock. J. Environ. Hort. 16: 202–207.

Oliver, J.B., M.E. Reding, S.O. Dennis, J.J. Moyseenko, N.N. Youssef, M.G. Klein, A. Callcott, S.S. James, L.R. McAnally and B.L. Bishop. 2008. Drench treatments for management of larval Japanese beetle (Coleoptera: Scarabaeidae) in field-grown balled and burlapped nursery plants. J. Econ. Entomol. 101: 1158–1166.

Oliver, J.B., M.E. Reding, M.G. Klein, N.N. Youssef, C.M. Mannion, B. Bishop, S.S. James and A. Callcott. 2007. Chlorpyrifos immersion to eliminate third instars of Japanese beetle (Coleoptera: Scarabaeidae) in balled and burlapped trees and subsequent treatment effects on red maple. J. Econ. Entomol. 100: 307–314.

Oliver, J.B., M.E. Reding, C.M. Ranger, M.G. Klein, N.N. Youssef, J.J. Moyseenko and S.O. Dennis. 2016. Insecticides evaluated as regulatory immersion treatments to eliminate third-instar Japanese beetles (Coleoptera: Scarabaeidae) from small-diameter field-grown nursery plants. J. Entomol. Sci. 51: 9–28.

Oliver, J.B., M.E. Reding, N.N. Youssef, M.G. Klein, B.L. Bishop and P.A. Lewis. 2009. Surface-applied insecticide treatments for quarantine control of Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), larvae in field-grown nursery plants. Pest Manag. Sci. 65: 381–390.

Potter, D.A. and D.W. Held. 2002. Biology and management of Japanese beetle. Annu. Rev. Entomol. 47: 175–205.

Shetlar, D.J. and J. Andon. 2012. Identification of white grubs in turfgrass. The Ohio State University Entomology Factsheet. HYG-2510-12. The Ohio State Univ., Columbus, OH.

Simmons, L.D. and J.F. Derr. 2007. Pendimethalin movement through pine bark compared to field soil. Weed Technol. 21: 873–876.

Smitley, D.R. 1994. Dealing with Japanese beetle: A new approach. Nursery Business Grower. August: 6–7.

USDA Animal and Plant Health Inspection Service. 2007. Managing the Japanese Beetle: A Homeowner's Handbook. USDA-APHIS Program Aid No. 1599.

Wilkinson, C.F. 1976. Insecticide interactions, Pp. 605–647. *In* Wilkinson, C.F. (ed.), Insecticide Biochemistry and Physiology. Springer US, New York.